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Dietary fat and hormonal influences on lipoprotein fluidity and composition in premenopausal women

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Summary

LDL and HDL became more fluid when healthy, free-living, premenopausal women were fed reduced fat diets with higher proportions of polyunsaturated fatty acids. Lipoproteins were isolated from plasma of 31 female subjects fed one of two sets of diets from typical U.S.A. foods with P/S ratios of 0.3 or 1.0. All subjects were fed high-fat diets (40% of energy) for the duration of four menstrual cycles followed by low-fat diets (20% of energy) for the next four cycles. Blood samples were collected during mid-follicular and mid-luteal phases of the fourth menstrual cycle of each diet period to assess interactive dietary and hormonal control of lipoprotein fluidity. LDL was significantly more fluid, as determined by DPH fluorescence, upon reducing fat consumption from 40 to 20% of energy for subjects eating foods with P/S = 1.0 or 0.3. Generally LDL was more fluid during the follicular phase than the luteal phase of the cycles, thus indicating hormonal influences on LDL fluidity. HDL results were similar but not as pronounced as with LDL. Lipoprotein phospholipid (PL) and cholesteryl ester (CE) fatty acyl compositions were also subject to dietary and hormonal influences. Effects were noted in several fatty acids depending upon diet and hormonal state; however, generally diet fat reduction resulted in reduced linoleate and increased oleate contents. Regression analyses showed that fluidity was more dependent upon the lipoprotein cholesterol content than upon fatty acyl composition.

Key words: Dietary fat; Hormonal influence; Lipoproteins; Lipoprotein composition; Premenopausal women

Introduction

Dietary fatty acid saturation level is generally known to influence plasma lipoprotein composi-

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tion and physical chemical properties in males, but relatively few data are available for women. Indeed atherosclerosis research has been directed primarily at men, since premenopausal women have a much lower incidence of arteriosclerosis than males of the same age. Under age fifty, 90% of arteriosclerotic events occur in men [1], but after menopause women appear to lose their advantage, with the incidence of myocardial infarction similar for both sexes [2–4] in the sixth and seventh decades of life. The protective agent in the younger women is thought to be estrogen, and indeed increases in atherosclerotic risk parallel reductions in endogenous secretion of the hormone.

High density lipoprotein cholesterol (HDL-C) levels, an important negative risk factor for coronary heart disease [6], are generally greater in women than in men [7,8], and exogenous estrogens [9] are known to affect HDL-C levels; hence it is thought that hormone-related protection of women from heart disease operates through control of HDL-C levels. Hormonal control of lipoprotein cholesterol levels has been reported [10–12], with HDL-C higher in premenopausal women than in adult men and low density lipoprotein cholesterol (LDL-C) lower in the women. We have demonstrated a sex-related difference in lipid phase fluidity in HDL from miniature swine [13]. Following puberty HDL became progressively more rigid with age in the male minipig but not in the female despite identical diets. We have reported [14] that moderate increases in dietary linoleate in men increased LDL fluidity, apparently through control of LDL phospholipid fatty acyl unsaturation. There have been studies [15–18] in women of dietary fat modification, i.e. percent fat and P/S ratio, effects on plasma lipids, but only one study [19], with a few subjects, of modulation of lipoprotein fluidity in women. More fluid lipoproteins may offer some protection against heart disease by affecting lipoprotein/receptor interactions and/or by modulating reverse cholesterol transport by HDL. In the present work we studied dietary and hormonal status effects on lipoprotein composition and fluidity in adult women by feeding high-fat, 40% of energy, and low-fat, 20% of energy, diets with P/S ratios of 0.3 and 1.0 and examining lipoproteins from blood samples taken during the follicular and luteal phases of the menstrual cycle.

Materials and methods *

Subjects and experimental diets

Premenopausal women aged 20–40 years were recruited from the Beltsville, MD area to participate in a study of the effects on various biologic parameters [20–24] of eating high-fat (40% of energy) versus low-fat (20% of energy) diets at low, 0.3, or high, 1.0, P/S, ratios of polyunsaturated to saturated fatty acids. Ninety-seven volunteers were first screened as potential subjects to be sure that there was no history of metabolic or chronic disease, no regular medications including oral contraceptives, no menstrual irregularities, no current or recent (1 year) pregnancy or lactation and no unusual dietary pattern, e.g. vegetarian. Forty women meeting these criteria were then further screened with a more detailed medical history, physical examination and laboratory tests to ensure good health. Subjects whose weights were less than 90% or greater than 120% of the 1983 Metropolitan Life Insurance 'desirable weights' [25] were excluded. Thirty-seven women passed the screening and entered the study but only 31 subjects completed the entire nine-month study. The data presented here represent only the 31 women who completed the study. All the procedures were approved by the Human Studies Committees of the U.S. Department of Agriculture, the National Institutes of Health and the Georgetown University Medical School.

Subjects were paired on relative weight basis (weight/height) and randomized to one of two dietary groups, $P/S = 0.3$ or $P/S = 1.0$, which were maintained throughout both the high- and low-fat dietary regimens. Smokers ($n = 7$) were evenly represented in both P/S groups. After a pre-diet free-choice period lasting one menstrual

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cycle, the women were placed on the high-fat diets for four menstrual cycles and then switched to the low-fat diets for a similar period of four menstrual cycles. Thus, 16 women consumed diets with 40% of energy from fat, P/S = 1.0, from October/November until February/March and diets with 20% of energy from fat, P/S = 1.0, from February/March until May/July, while the other 15 women concurrently ate diets with 40% of energy from fat, P/S = 0.3, from October/November until February/March and diets with 20% of energy, P/S = 0.3, from February/March until May/July. There was no break between the dietary periods. The variation in starting and finishing dates for the dietary regimens reflects the individual menstrual cycles of the subjects. Thus in all data presentations in this paper $n = 31$ for data from the self-selected prestudy period, $n = 16$ for data for subjects eating P/S = 1.0 diets and $n = 15$ for data from subjects eating P/S = 0.3 diets.

Diets contributing either 40% or 20% energy from fat with P/S ratios of 0.3 and 1.0 were formulated from commonly available foods. Nutrient compositions of the diets were calculated using the Lipid Nutrition Laboratory database derived from data on food composition from the USDA, the food industry, the Nutrient Coding Center in Minneapolis, and by analysis. A 14-day menu cycle was used to provide variety and maintain acceptability of the diets. Caloric intakes were adjusted, within the constraints of the dietary formulations, to maintain constant body weight. All nutrients for which food data are available were provided by the diets in amounts needed to meet the Recommended Dietary Allowances [26]. Reduction of energy from fat was compensated for by increasing carbohydrate content, but with the ratio of complex to simple sugars maintained at approximately 1:1. Protein levels were held at 16–17% of energy. The mean daily dietary intake for the two P/S groups during the high- and low-fat periods is shown in Table 1.

The controlled diets were prepared in the Human Studies Facility of the Beltsville Human Nutrition Research Center (BHNRC). Weekday morning and evening meals were eaten in the BHNRC dining facility, and a carry-out lunch was provided. Meals for Saturdays, Sundays and holidays were prepacked and distributed for home consumption. Alcohol consumption was not allowed during the controlled diet study.

Blood sampling and lipoprotein isolation

Morning fasting blood samples were collected during two successive 5-day periods corresponding to mid-follicular and mid-luteal phases of the menstrual cycle, as estimated from menses dates, during the pre-study 'baseline' period and the fourth cycle of each experimental diet period. Estimates of the times for the follicular and luteal phases of the cycle were corroborated by monitoring urinary and plasma sex hormone levels [27,28]. Disodium EDTA was used as anticoagulant. Blood cells were removed by low-speed centrifugation (2250 × *g*, 15 min, 4°C), and chylomicrons and

TABLE 1

AVERAGE NUTRIENT COMPOSITIONS OF THE EXPERIMENTAL DIET (DAILY INTAKES AS MEANS ± SEM)

Nutrient	Fat (% of energy)			
	40%		20%	
P/S ratio:	0.3	1.0	0.3	1.0
Energy (kcal)	2278 ± 65	2180 ± 81	2260 ± 97	2208 ± 110
Protein (% kcal)	16	16	17	17
Carbohydrate (% kcal)	45	45	64	64
Fat (% kcal)	39	39	19	19
Cholesterol (mg)	374 ± 11	289 ± 10	230 ± 9	199 ± 9
Saturated fat (g)	44.2 ± 1.3	26.8 ± 1.1	20.9 ± 0.7	12.3 ± 0.6
Oleic acid (g)	30.5 ± 0.9	33.5 ± 1.4	14.9 ± 0.6	17.0 ± 0.8
Linoleic acid (g)	14.6 ± 0.4	26.1 ± 0.9	6.9 ± 0.2	12.9 ± 0.6

other large particles ($S_f > 400$) were removed by flotation (26,000 × g, 30 min, ambient temperature). The major lipoprotein fractions were isolated from the chylomicron-free plasma by sequential ultracentrifugal flotation using standard techniques [29,30]. Plasma or plasma diluted with 0.195 molal NaCl ($\rho = 1.006$ g/ml) was centrifuged for 18 h at 40,000 rpm in a Beckman ultracentrifuge (40.3 rotor) to concentrate VLDL at the tops of the centrifuge tubes. After removal of the first ml containing the VLDL and a second reference ml, the 4 ml infranatant was mixed with 2 ml of a NaCl/NaBr solution ($\rho = 1.182$ g/ml) and centrifuged for an additional 18 h to concentrate LDL, the fraction floating at $\rho = 1.063$ g/ml, at the tops of the tubes. After removal of the first ml containing the LDL and a second reference ml, the 4 ml infranatant was mixed with 2 ml of a NaCl/NaBr solution ($\rho = 1.478$ g/ml) and centrifuged for 24 h to concentrate the HDL fraction floating at $\rho = 1.216$ g/ml at the tops of the centrifuge tubes. To minimize losses, syringes and cannulas were rinsed with 0.195 molal NaCl which was added to the isolates. Lipoprotein fractions as isolated were in the first ml, as the second or reference ml was usually lipid-free. Lipoprotein recoveries were assessed by comparing the sums of VLDL, LDL and HDL cholesterols with the plasma cholesterols and by comparing these results with data obtained with the microfractionation method of Bronzert and Brewer [31]. Lipoprotein recoveries averaged 90% and the calculated individual recoveries were factored into the lipid analytical data presented in this paper.

Fluidity measurements

Lipoprotein fluidity was assessed as a function of temperature between 20° and 40°C by determining the anisotropy of fluorescence from the probe 1,6-diphenyl-1,3,5-hexatriene (DPH) using the methods of Shinitzky and Barenholz [32]. DPH (2 mM in tetrahydrofuran) was diluted (1000-fold) into the aqueous lipoprotein solution, which was then incubated with agitation at 35–37°C for 2 h.

Steady-state fluorescence polarization intensity was measured with an Aminco-Bowman spectrophotofluorometer equipped with Glan-Thompson prism polarizers. Excitation and emission wavelengths were 366 and 450 nm respectively. The measured anisotropies were obtained from the intensities of emission polarized parallel and perpendicular to the polarized excitation by the use of standard formulae including an appropriate grating correction [33,34]. Light scattering errors were minimized by ensuring that the measured anisotropies were concentration-independent.

Chemical analysis

Plasma and lipoprotein cholesterol and cholesteryl ester contents were determined enzymatically [35] using cholesterol oxidase alone for free cholesterol analysis and in combination with cholesterol esterase for total cholesterol analysis. Triacylglycerol was determined enzymatically [36], and chemical methods were used for protein [37] and phospholipid [38] analyses. Enzymatic analyses for cholesterol and triacylglycerol were repeatedly standardized with pure cholesterol and triolein (Sigma). Analyses were performed in triplicate with standard curves redetermined for every 40 samples.

Fatty acyl compositions of the LDL and HDL phospholipid and cholesteryl ester fractions were determined by gas chromatography [39]. Lipids were extracted with $CHCl_3/CH_3OH$ by an adaptation [39] of the method of Sperry and Brand [40], and the total lipids were separated into two fractions consisting of neutral lipids and phospholipids essentially as described by Rouser et al. [41]. The total lipid, dissolved in a minimum volume of chloroform (less than 500 μl), was applied to a column containing 0.5 g Unisil (Clarkson Chemical Co.), and $CHCl_3$ (15 ml) and CH_3OH (20 ml) were used to elute the neutral and polar lipid fractions, respectively. The cholesteryl ester fraction was separated from other neutral lipids by thin-layer chromatography on silica gel HR plates using hexane/diethyl ether/acetic acid (70 : 30 : 1) as the developing solvent. Studies with pure materials and test mixtures confirmed the accuracy, efficiency, completeness and reproducibility of the separations. Fatty acid methyl esters for gas chromatography were prepared by transesterification with methanolic HCl. Heptadecanoic acid was included with the samples as an internal standard. Chromatography was performed with a Hewlett-Packard Model 5700A gas chromatograph coupled to a Model 3885A auto-

mation system. The instrument was equipped with dual flame ionization detectors and a Model 7671A automatic sampler. Detection was in the dual differential mode. Stainless-steel columns (1.8 m) were packed with Supelcoport (Supelco) coated with a slurry of 10% H_3PO_4 modified ethylene glycol succinate [39].

Statistical analyses

Data were subjected to analysis of variance, linear regression analysis and the calculation of correlation coefficients for the various measurements. The model included sources of variation due to diet changes in fat and unsaturation level and hormonal state changes. Duncan's multiple range test was used to determine differences in model classified, composition and anisotropy data. All statistical analyses were performed using the computer methodology of the Statistical Analysis System [42].

Results and discussion

Steady-state DPH fluorescence anisotropies, r_s, at 37°C are presented in Table 2 as mean values for lipoprotein samples obtained from the subjects during the initial cycle, when they were eating diets of their own choice, and during the fourth menstrual cycle of each experimental diet period. Values are shown separately for the follicular and luteal phases of the cycles. Generally VLDL was more fluid when the subjects ate the higher-fat diets, but only significantly so for the luteal phase samples. Lowering fat intake significantly increased LDL fluidity in both P/S groups during both phases of the menstrual cycle. Luteal-phase data showed that HDL became significantly more fluid when dietary fat was lowered from 40 to 20% of energy for women eating P/S = 1.0 diets. Fat level, however, had no effect on HDL fluidity in the P/S = 0.3 group during the luteal phase. HDL tended to be more fluid upon lowering fat, though not significantly, in both groups of women during the follicular phase. In general lipoprotein fluidities observed following the self-selected diet period were similar to values obtained following the 40% fat periods, the only exception being VLDL in the follicular phase. These are statistically valid comparisons in that they correspond to changes in the same women upon lowering fat; but upon comparing the groups we noted that VLDL was more fluid for the P/S = 1.0 group during the follicular phase, and LDL was most fluid for subjects consuming the low-fat, high-P/S diet with r_s significantly lower than observed with women eating the 40% fat, P/S = 0.3 diet. Clearly, both diet and hormonal status influence lipoprotein fluidity, with the low-fat (20% of energy) high polyunsaturated fat (P/S = 1.0) diet yielding the most fluid lipoproteins.

TABLE 2

DPH FLUORESCENCE ANISOTROPY, r_s, AT 37°C IN THE SEVERAL LIPOPROTEIN FRACTIONS (MEANS± SEM)

Lipoprotein	Diet (energy %, P/S)	Cycle phase	
		Follicular	Luteal
VLDL	Self-selected	0.095 ± 0.003^{b}	0.094 ± 0.002^{b}
	40, 0.3	0.112 ± 0.008^{a}	0.092 ± 0.003^{b}
	20, 0.3	0.115 ± 0.007^{a}	0.115 ± 0.005^{a}
	40, 1.0	0.095 ± 0.005^{b}	0.097 ± 0.003^{b}
	20, 1.0	0.102 ± 0.005^{ab}	0.110 ± 0.006^{a}
LDL	Self-selected	0.208 ± 0.002^{a}	0.212 ± 0.002^{a}
	40, 0.3	0.209 ± 0.002^{a}	0.213 ± 0.002^{a}
	20, 0.3	0.193 ± 0.003^{bc}	0.197 ± 0.004^{b}
	40, 1.0	0.204 ± 0.008^{ab}	0.205 ± 0.003^{a}
	20, 1.0	0.190 ± 0.003^{c}	0.196 ± 0.004^{b}
HDL	Self-selected	0.197 ± 0.002^{a}	0.196 ± 0.002^{ab}
	40, 0.3	0.199 ± 0.003^{a}	0.194 ± 0.003^{ab}
	20, 0.3	0.191 ± 0.003^{ab}	0.193 ± 0.003^{ab}
	40, 1.0	0.195 ± 0.003^{ab}	0.199 ± 0.003^{a}
	20, 1.0	0.187 ± 0.004^{b}	0.188 ± 0.003^{b}

[abc] Values with different superscripts in a column for a lipoprotein are significantly different

Regression analyses demonstrated limited relationships between dietary unsaturates and fluidity expressed as DPH anisotropy. Fluidity in VLDL was related to oleate and linoleate intakes according to the following equations: $r_s = 0.120 - 7.04 \times 10^{-4}$ (g 18 : 1/*d*) with $P < 0.0001$ and $r = 0.3184$ and $r_s = 0.114 - 8.16 \times 10^{-4}$ (g 18 : 2/*d*) with $P < 0.0003$ and $r = 0.2641$. Fluidities in LDL and HDL, however, showed inverse relations to oleate and linoleate intakes, indicating that dietary control of lipoprotein fluidity is much more complex than merely involving the mass of unsaturated fatty acid ingested. DPH anisotropy in LDL was significantly related to dietary cholesterol (C) according to the equation: $r_s = 0.192 + 3.7 \times 10^{-5}$

(mg C/d) with $P < 0.0001$ when all LDL data are combined. Similar relations were obtained for LDL data for each of the menstrual phases, thus indicating that dietary cholesterol reduces LDL fluidity. No statistically significant relations were observed between HDL-DPH anisotropy and dietary cholesterol. VLDL-DPH anisotropy was inversely related ($P < 0.003$) to dietary cholesterol, indicating that control of VLDL fluidity is at a more complex level. The modifications in dietary lipids did, however, induce changes in lipoprotein composition, including cholesterol and cholesteryl ester contents and fatty acyl composition of the phospholipid and cholesteryl ester (CE) fractions.

The major change in lipoprotein fluidity observed in this study was in the DPH anisotropy as indicated in the 37°C data in Table 2. Thermotropic studies of DPH anisotropy exhibited monophasic behavior in the range 20–40°C; however, changes were observed in the temperature dependence of fluidity. Slope values for Arrhenius plots of r_s versus $1/T$ for each lipoprotein fraction, at the various dietary and hormonal states, are given in Table 3. Higher P/S levels tended to increase the temperature effects in VLDL though not always significantly. Hormonal state was important in the LDL fraction, with diet modulating slopes in the follicular-phase data but showing no effects with the luteal-phase data. Lower fat (20% of energy) increased the temperature modulation of LDL fluidity during the follicular phase for both groups of subjects but only significantly for those eating P/S = 1.0 diets. No significant effects on the HDL slope values were observed under any experimental conditions. The only significant change in VLDL was in reduction of thermotropic effects on VLDL isolated during the luteal phase upon lowering fat level for women eating P/S = 0.3 diets.

TABLE 3

EFFECT OF TEMPERATURE ON DPH FLUORESCENCE ANISOTROPY AS ARRHENIUS PLOT SLOPES, m, WHERE $\ln r_s = \ln A + m\ (1/T)$ FOR THE SEVERAL LIPOPROTEIN FRACTIONS

Values are means (±SEM) for $m \times 10$

Lipoprotein	Diet (energy %, P/S)	Cycle phase	
		Follicular	Luteal
VLDL	Self-selected	2.28±0.04 [a]	2.33±0.05 [a]
	40, 0.3	1.95±0.16 [b]	2.26±0.10 [a]
	20, 0.3	1.96±0.10 [b]	1.95±0.10 [b]
	40, 1.0	2.13±0.09 [ab]	2.19±0.09 [ab]
	20, 1.0	2.14±0.14 [ab]	2.08±0.08 [ab]
LDL	Self-selected	1.41±0.03 [b]	1.45±0.03 [a]
	40, 0.3	1.41±0.04 [b]	1.39±0.03 [a]
	20, 0.3	1.51±0.08 [ab]	1.42±0.07 [a]
	40, 1.0	1.41±0.04 [b]	1.50±0.05 [a]
	20, 1.0	1.59±0.05 [a]	1.47±0.06 [a]
HDL	Self-selected	1.62±0.03 [a]	1.60±0.03 [a]
	40, 0.3	1.59±0.04 [a]	1.62±0.07 [a]
	20, 0.3	1.60±0.06 [a]	1.63±0.04 [a]
	40, 1.0	1.57±0.06 [a]	1.50±0.05 [a]
	20, 1.0	1.65±0.08 [a]	1.56±0.06 [a]

[ab] Values with different superscripts in the same column for a lipoprotein are significantly different.

TABLE 4

EFFECT OF DIET AND HORMONAL STATE ON PLASMA CHOLESTEROL AND TRIACYLGLYCEROL LEVELS (mg/dl)

Diet (energy %, P/S)	Cholesterol		Triacylglycerol	
	Follicular phase	Luteal phase	Follicular phase	Luteal phase
40, 0.3	160.1±8.2	148.4±7.4	37.2±2.7 [b]	43.2±5.6
20, 0.3	157.3±8.5	152.2±7.7	51.3±4.6 [ab]	48.8±4.0
40, 1.0	151.9±6.7	139.8±6.1	43.0±5.1 [ab]	41.2±3.8
20, 1.0	153.8±6.6	149.1±8.0	57.2±9.8 [a]	53.8±7.4

[ab] Follicular-phase triacylglycerol values with different superscripts are significantly different. No statistically significant differences were noted in the values in any other column.

Plasma total cholesterol levels (Table 4) were not affected by diet, but plasma triacylglycerol levels showed non-significant increases on lowering dietary fat percentage at either P/S level. The only significant difference was noted when comparing data for the 40% fat, P/S = 0.3 diet with the 20% fat, P/S = 1.0 diet, but this comparison involves different subjects. The alteration in plasma triacylglycerol levels may be related to increased lipogenesis induced by the higher carbohydrate content of the 20% fat diet. Dietary carbohydrate influences on lipid metabolism have been reported [43–45]. Bhathena et al. [22] observed elevated insulin levels when the subjects ate the lower-fat (high carbohydrate) diets. Though higher insulin levels might be associated with in-

TABLE 5

EFFECT OF DIET AND HORMONAL STATE ON LDL AND HDL CHOLESTEROLS (mg/dl)

Lipoprotein	Diet (energy %, P/S)	Follicular phase		Luteal phase	
		Free cholest.	Ester. cholest.	Free cholest.	Ester. cholest.
LDL	40, 0.3	37.4 ± 4.1 [a]	67.2 ± 7.9 [a]	40.8 ± 3.9 [a]	57.8 ± 4.6 [a]
	20, 0.3	23.6 ± 2.6 [b]	66.6 ± 6.5 [a]	19.7 ± 1.5 [c]	57.1 ± 6.3 [a]
	40, 1.0	33.7 ± 2.2 [a]	58.9 ± 3.8 [a]	31.5 ± 2.6 [b]	54.7 ± 3.9 [a]
	20, 1.0	17.8 ± 2.2 [b]	58.1 ± 3.0 [a]	22.3 ± 2.9 [c]	58.1 ± 3.3 [a]
HDL	40, 0.3	14.2 ± 1.8 [a]	38.6 ± 4.1 [b]	15.0 ± 2.1 [a]	32.6 ± 4.0 [c]
	20, 0.3	18.5 ± 2.1 [a]	46.8 ± 3.3 [ab]	17.2 ± 2.1 [a]	55.3 ± 2.5 [a]
	40, 1.0	17.7 ± 2.1 [a]	39.6 ± 4.3 [b]	15.8 ± 1.4 [a]	35.2 ± 2.2 [c]
	20, 1.0	18.5 ± 1.9 [a]	56.2 ± 4.6 [a]	21.0 ± 2.9 [a]	44.5 ± 3.3 [b]

[ab] Values in a column for a lipoprotein with different superscripts are significantly different.

creased lipogenesis, the total spectrum of metabolic regulatory hormones must be taken into account. Plasma insulin levels were elevated during both menstrual phases, but increases in plasma triacylglycerol levels were less pronounced during the luteal phase. Apparently estrogen secretion during the follicular phase of the menstrual cycle resulted in a more pronounced mobilization of

TABLE 6

EFFECT OF DIETARY FAT REDUCTION ON LDL PHOSPHOLIPID FATTY ACYL COMPOSITION, FOLLICULAR PHASE DATA (FATTY ACID MOL PERCENTAGES AND U/S AND P/S RATIOS ARE MEAN VALUES)

Acid	P/S:	0.3		1.0	
	% Energy:	40.0	20.0	40.0	20.0
12:0		0.11 [b]	0.34 [a]	0.18	0.25
14:0		0.82 [b]	1.42 [a]	0.79 [a]	1.09 [b]
15:0		0.64	0.49	0.41	0.39
16:0		33.64	32.11	32.40	33.85
16:1		1.90	4.61	2.01 [b]	2.65 [a]
18:0		14.85	13.28	15.74 [a]	13.79 [b]
18:1		15.00 [b]	18.76 [a]	16.05 [b]	20.50 [a]
18:2		19.44 [a]	15.82 [b]	19.25	16.34
18:3		0.45	1.80	0.49 [b]	0.66 [a]
20:0		0.85	0.76	0.84 [a]	0.66 [b]
20:3		3.53 [a]	2.65 [b]	3.25 [a]	2.15 [b]
20:4		2.26 [a]	1.57 [b]	1.42	1.32
20:5		0.59	0.81	0.72	0.74
22:5		0.61	0.28	0.83	0.60
22:6		0.52	0.51	0.87	0.64
24:0		2.19	1.98	2.37 [a]	1.84 [b]
24:1		2.79	2.76	2.38	2.48
U/S [c]		0.89	0.90	0.91	0.94
P/S		0.52 [a]	0.42 [b]	0.52	0.44

[ab] Values for a fatty acid or ratio with different superscripts for a P/S group are significantly different, thus indicating a change in the mole percent of that fatty acid or the mole percent ratio when fat intake is lowered from 40 to 20% of energy. Superscripts are omitted when there are no significant effects.

[c] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated fatty acids.

triacylglycerols from depot fat when the subjects ate less fat. Diet did not affect plasma cholesterol levels, but there was an apparent cycle effect, with plasma cholesterol lower during the luteal phase than during the follicular phase of the cycle regardless of diet treatment. Jones et al. [20] have reported on this effect elsewhere and suggested that the changes correspond to alterations in LDL cholesterols. Estrogen-induced lowering of cholesterol in rats has been reported and has been associated with increased cholesterol catabolism in the liver [46]. Cyclic reductions in plasma cholesterol may result from estrogen modulation of LDL binding to its receptor. Kovanen et al. [47] demonstrated increased LDL binding by hepatocyte membranes from rats treated with 17α-ethinyl estradiol.

Free and esterified cholesterol data are given in Table 5 for both LDL and HDL. Lowering fat intake reduced LDL free cholesterol but had no effect on LDL cholesterol ester content regardless of P/S level or menstrual cycle phase. LDL free cholesterol was significantly higher for the women eating the 40% fat, P/S = 0.3 diet than any other during the luteal phase of the cycle. During both phases of the menstrual cycle HDL-CE content was higher for the lower-fat diets while HDL free cholesterol was not affected. HDL-CE was significantly higher at the 20% fat P/S = 1.0 diet than any other. This may reflect an enhanced ability of the HDL to transport cholesteryl ester from peripheral tissue for catabolism when a diet high in polyunsaturates is consumed. Mechanisms have been proposed [48] whereby more fluid HDL has an enhanced capacity for 'reverse cholesterol transport'.

LDL phospholipid fatty acyl compositions are given as mole percentages in Tables 6 and 7 for

TABLE 7

EFFECT OF DIETARY FAT REDUCTION ON LDL PHOSPHOLIPID FATTY ACYL COMPOSITION, LUTEAL-PHASE DATA (FATTY ACID MOL PERCENTAGES AND U/S AND P/S RATIOS ARE MEAN VALUES)

Acid	P/S:	0.3		1.0	
	% Energy:	40.0	20.0	40.0	20.0
12:0		0.14 [b]	0.26 [a]	0.12	0.16
14:0		0.97 [b]	1.29 [a]	0.74 [b]	1.08 [a]
15:0		0.55	0.47	0.37	0.38
16:0		36.07	34.15	32.11	33.83
16:1		2.08 [b]	2.66 [a]	1.78 [b]	2.89 [a]
18:0		15.55 [a]	13.94 [b]	19.98	13.81
18:1		16.80 [b]	19.18 [a]	15.44 [b]	22.26 [a]
18:2		15.04	15.85	20.40 [a]	15.17 [b]
18:3		0.47 [b]	0.58 [a]	1.89	0.67
20:0		0.91 [a]	0.74 [b]	0.80	0.74
20:3		2.73	2.68	3.21 [a]	1.89 [b]
20:4		1.32	1.88	1.65 [a]	0.97 [b]
20:5		0.51 [b]	0.79 [a]	0.73	0.70
22:5		1.39	0.64	1.09	0.39
22:6		0.71 [a]	0.33 [b]	0.65	0.73
24:0		2.16	1.85	2.24 [a]	1.87 [b]
24:1		2.59	2.70	2.37	2.48
U/S [c]		0.78 [b]	0.91 [a]	0.91	0.95
P/S		0.40	0.44	0.54 [a]	0.41 [b]

[ab] Values for a fatty acid or ratio with different superscripts for a P/S group are significantly different, thus indicating a change in the mole percent of that fatty acid or the mole percent ratio when fat intake is lowered from 40 to 20% of energy. Superscripts are omitted when there are no significant effects.

[c] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated fatty acids.

TABLE 8

EFFECT OF DIETARY FAT REDUCTION ON HDL PHOSPHOLIPID FATTY ACYL COMPOSITION, FOLLICULAR-PHASE DATA (FATTY ACID MOL PERCENTAGES AND U/S AND P/S RATIOS ARE MEAN VALUES)

Acid	P/S:	0.3		1.0	
	% Energy:	40.0	20.0	40.0	20.0
12:0		0.24	0.15	0.30 [a]	0.11 [b]
14:0		1.19	1.05	1.09 [a]	0.81 [b]
15:0		0.32 [b]	0.48 [a]	0.33	0.35
16:0		32.57 [b]	35.82 [a]	31.12	31.92
16:1		1.46 [b]	2.32 [a]	1.36 [b]	1.83 [a]
18:0		16.14 [b]	17.72 [a]	16.22	16.61
18:1		14.26 [b]	17.51 [a]	14.17 [b]	15.43 [a]
18:2		21.13 [a]	14.94 [b]	22.20	20.32
18:3		0.54	0.52	0.53	0.53
20:0		0.57	0.74	0.54	0.55
20:3		2.49	2.10	2.52	2.69
20:4		3.88 [a]	1.91 [b]	4.03	3.13
20:5		1.05	0.96	1.12	1.26
22:5		0.45	0.59	0.45	0.61
22:6		0.85 [a]	0.29 [b]	1.00	0.60
24:0		1.48	1.30	1.53	1.52
24:1		1.38 [b]	1.85 [a]	1.48 [b]	1.75 [a]
U/S [c]		0.92	0.76	0.97	0.93
P/S		0.59 [a]	0.38 [b]	0.64	0.57

[ab] Values for a fatty acid or ratio with different superscripts for a P/S group are significantly different, thus indicating a change in the mole percent of that fatty acid or the mole percent ratio when fat intake is lowered from 40 to 20% of energy. Superscripts are omitted when there are no significant effects.

[c] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated fatty acids.

the two phases of the menstrual cycle. The follicular-phase data (Table 6) show that lowering dietary fat significantly increased LDL phospholipid (PL) oleate (18:1) regardless of diet P/S. Constancy of LDL-PL U/S is maintained through compensatory increases in oleate and decreases in linoleate (18:2). The decrease in 18:2, however, was only statistically significant for the P/S = 0.3 group. The decrease in PL linoleate probably resulted from the reduced intake via the lower fat diets, while the increased oleate is likely from de novo synthesis or via desaturase activity from stearate, since dietary oleate was reduced in the low-fat diets (Table 1). The luteal-phase data (Table 7) show a similar significant increase in oleate regardless of dietary P/S, but the decrease in 18:2 is only seen for the P/S = 1.0 group. Stearate (18:0) decreased in both groups on lowering dietary fat but only significantly for the P/S = 0.3 group. This is opposite to the effects observed with PL stearate during the follicular phase. In the luteal phase lipoprotein U/S increased on lowering diet fat, significantly for the P/S = 0.3 group, but lipoprotein P/S decreased for the diet P/S = 1.0 group only.

HDL phospholipid fatty acyl compositions are given in Tables 8 and 9 for the proliferative and secretory phases of the menstrual cycle. The proliferative-phase data (Table 8) show significant increases in stearate and oleate and significant decreases in linoleate and arachidonate for the P/S = 0.3 group but only significant increases in oleate for the P/S = 1.0 group. The HDL-PL follicular-phase data show significant decreases in U/S and P/S for the P/S = 0.3 group on lowering fat intake but no such changes were noted with the women eating the P/S = 1.0 diet. The luteal-phase data for HDL PL (Table 9) show very little change on lowering fat intake. The U/S and P/S ratios were not affected, and the only fatty

TABLE 9

EFFECT OF DIETARY FAT REDUCTION ON HDL PHOSPHOLIPID FATTY ACYL COMPOSITION, LUTEAL-PHASE DATA (FATTY ACID MOL PERCENTAGES AND U/S AND P/S RATIOS ARE MEAN VALUES)

Acid	P/S:	0.3		1.0	
	% Energy:	40.0	20.0	40.0	20.0
12:0		0.32	0.24	0.37 [a]	0.14 [b]
14:0		1.18	1.06	1.35 [a]	0.82 [b]
15:0		0.39	0.47	0.37	0.34
16:0		33.74	34.02	30.70	32.40
16:1		1.59	2.07	1.65	1.92
18:0		16.66	16.43	17.14	17.01
18:1		14.70	15.95	14.41 [b]	16.23 [a]
18:2		19.58	18.66	22.72 [a]	18.93 [b]
18:3		0.51	0.54	0.56	0.57
20:0		0.59	0.56	0.62	0.57
20:3		2.36	2.67	2.31	2.66
20:4		3.26	3.21	2.84	2.82
20:5		1.29	0.94	1.08	1.24
22:5		0.34	0.23	0.23 [b]	0.47 [a]
22:6		0.75	0.41	0.68	0.69
24:0		1.56 [a]	0.98 [b]	1.66	1.46
24:1		1.76	1.69	1.33 [b]	1.73 [a]
U/S [c]		0.86	0.87	0.92	0.91
P/S		0.53	0.50	0.59	0.53

[ab] Values for a fatty acid or ratio with different superscripts for a P/S group are significantly different, thus indicating a change in the mole percent of that fatty acid or the mole percent ratio when fat intake is lowered from 40 to 20% of energy. Superscripts are omitted when there are no significant effects.

[c] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated fatty acids.

acyl changes were increased 18:1 and lowered 18:2 for the women eating the P/S = 1.0 diets. The differences in dietary effects seen on comparing the data in Tables 8 and 9 do suggest that there is some hormonal control of HDL composition.

LDL cholesteryl ester fatty acyl compositions are given in Tables 10 and 11 for the follicular- and luteal-phase data respectively. The level of unsaturation (U/S values) in the CE fraction is substantially higher than in the PL fraction. The follicular-phase data (Table 10), regardless of diet P/S, show increased 16:0 and 16:1 and decreased 18:2 contents on lowering fat intake. Stearate and oleate were not affected. U/S ratios were significantly decreased at the lower-fat diet, but P/S was significantly decreased with the lower-fat diet for the dietary P/S = 1.0 group only. The luteal-phase data (Table 11) show almost no change for the P/S = 0.3 group on lowering fat, but the P/S = 1.0 group data show substantial decreases in unsaturation, reflecting decreased 18:2 and increased 16:0 apparently not offset by the higher 18:1.

HDL CE fatty acyl compositions are given in Tables 12 and 13 for the two cycle phases. There is a distinct cycle effect, as significant decreases in both U/S and P/S are seen regardless of diet P/S when fat intake is lowered in the follicular phase but not the luteal-phase data. The change in unsaturation in the follicular-phase data reflects increased 16:0 and decreased 18:2. The major effect of reducing fat intake during the luteal phase was to increase oleate content in the HDL CE fraction. There were also some changes in the longer-chain polyunsaturates (22:5 and 22:6) in the P/S = 0.3 group.

A hormonal status effect was noted in the linoleate content of the various fractions upon consumption of the 40% fat, P/S = 0.3 diet. The

relative mole percentage of 18 : 2 was always lower in the luteal phase compared to the follicular phase, in all lipid compartments examined (Tables 6–13). The drop was most pronounced in the HDL cholesteryl ester fraction.

The factors usually associated with lipid phase fluidity are fatty acyl unsaturation, cholesterol/phospholipid ratios, phospholipid headgroups, and triacylglycerol levels. Linear regression analyses were performed to determine if there were any significant relations between LDL or HDL fluidity expressed as DPH anisotropy and several parameters describing the level of unsaturation including PL-P/S and CE-P/S, PL-U/S and CE-U/S, and mole percents of 18 : 1, 18 : 2, 18 : 1 + 18 : 2. There were no consistent significant relationships between r_s in either LDL or HDL and any of these parameters whether all data were combined or segregated in terms of either dietary fat level, dietary P/S, or hormonal state. Linear relations were noted in a few combinations of diet and hormonal parameters; however, some of these were illogical inverse fluidity-unsaturation relations. No consistent relations emerged from the data, indicating that lipoprotein fatty acyl composition was not the determinant of either LDL or HDL fluidity in this study, in contrast with our previous work [14] showing a significant relation between LDL fluidity and LDL PL linoleate. Regression analysis did show a significant relationship between LDL free cholesterol content (LDL-FC) and DPH anisotopy according to the following equations: all data: $r_s = 0.192 + 0.00036$ (LDL-FC) with $P < 0.0001$ and $r = 0.348$; follicular: $r_s = 0.190 + 0.00041$ (LDL-FC) with $P < 0.002$, $r = 0.400$; luteal: $r_s = 0.194 + 0.00032$

TABLE 10

EFFECT OF DIETARY FAT REDUCTION ON LDL CHOLESTERYL ESTER FATTY ACYL COMPOSITION, FOLLICULAR-PHASE DATA (FATTY ACID MOL PERCENTAGES AND U/S AND P/S RATIOS ARE MEAN VALUES)

Acid	P/S:	0.3		1.0	
	% Energy:	40.0	20.0	40.0	20.0
12 : 0		0.12	0.04	0.01	0.02
14 : 0		1.39	1.76	1.08	1.41
15 : 0		0.59	0.70	0.51	0.50
16 : 0		30.53 [b]	34.79 [a]	28.08 [b]	35.27 [a]
16 : 1		4.19 [b]	6.06 [a]	4.14 [b]	5.54 [a]
18 : 0		6.02	4.79	3.58	4.18
18 : 1		25.60	29.40	25.51	28.12
18 : 2		24.83 [a]	16.52 [b]	28.73 [a]	18.77 [b]
18 : 3		0.74 [b]	0.95 [a]	0.74 [b]	1.04 [a]
20 : 0		0.21	0.34	0.08	0.24
20 : 3		0.20	0.13	0.17	0.15
20 : 4		0.56	0.30	0.59	0.36
20 : 5		0.30	0.27	0.40	0.36
22 : 5		3.16	2.33	4.81	2.08
22 : 6		1.22	1.44	1.06	1.67
24 : 0		0.34	0.17	0.35	0.17
24 : 1		0.02	0.01	0.17	0.13
U/S [c]		1.67	1.43	2.00 [a]	1.44 [b]
P/S		0.85 [a]	0.55 [b]	1.11 [a]	0.62 [b]

[ab] Values for a fatty acid or ratio with different superscripts for a P/S group are significantly different, thus indicating a change in the mole percent of that fatty acid or the mole percent ratio when fat intake is lowered from 40 to 20% of energy. Superscripts are omitted when there are no significant effects.

[c] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated fatty acids.

(LDL-FC) with $P < 0.02$, $r = 0.303$. There were no similar significant relations for HDL fluidity. We may conclude from these data that, at least for the LDL fraction, the cholesterol content was a more significant factor than fatty acyl unsaturation in the control of fluidity. This is compatible with our earlier reports that lipoproteins from rabbits fed corn oil were no more fluid than those from rabbits fed the highly saturated fat cocoa butter [49,50]. Though relationships between lipoprotein fluidity and fatty acyl unsaturation were not observed in this study we can conclude that a hypocholesterolemic low-fat, high-P/S, low-cholesterol diet does yield more fluid LDL and HDL fractions. This study has thus demonstrated that a hypocholesterolemic low-fat, high-P/S, low-cholesterol diet yields more fluid LDL and HDL fractions. It is expected that alterations in the physical state of plasma lipoprotein lipids would impinge upon interactions of proteins with these lipids. Such effects could either modulate apoprotein conformation on the LDL or HDL surface and thereby affect binding to lipoprotein receptors at various tissue sites or could affect the interactions of cell membrane proteins with lipoprotein lipids and thus directly affect binding of the lipoproteins to cells and thereby mediate tissue deposition of lipids as in atherogenesis. Soutar has suggested [51] that more fluid lipoprotein states may impinge upon the activity of lipoprotein receptors and thereby influence the atherosclerotic process. Ibdah and Philips [52] have studied the effects of lipid composition on the adsorption of apolipoprotein A-I to lipid monolayers as a model for the interactions of the apoprotein with the LDL or HDL lipid phase. They reported that binding was monolayer composition-dependent, with binding enhanced when the lipids were in an expanded rather than a condensed phase. Binding to the monolayer was reduced when it contained

TABLE 11

EFFECT OF DIETARY FAT REDUCTION ON LDL CHOLESTERYL ESTER FATTY ACYL COMPOSITION, LUTEAL-PHASE DATA (FATTY ACID MOL PERCENTAGES AND U/S AND P/S RATIOS ARE MEAN VALUES)

Acid	P/S:	0.3		1.0	
	% Energy:	40.0	20.0	40.0	20.0
12:0		0.10	0.20	0.01	0.01
14:0		1.71	1.75	1.04	1.24
15:0		0.74	0.67	0.44	0.49
16:0		33.73	33.68	29.98 [b]	34.18 [a]
16:1		4.44 [b]	5.60 [a]	3.85 [b]	5.33 [a]
18:0		4.10	3.71	3.17	3.79
18:1		28.43	29.84	25.95 [b]	29.13 [a]
18:2		19.69	18.36	28.86 [a]	19.76 [b]
18:3		0.88	0.93	0.79	0.87
20:0		0.11	0.21	0.08	0.23
20:3		0.15	0.13	0.55	0.13
20:4		0.47	0.31	0.25	0.32
20:5		0.35	0.40	0.40	0.31
22:5		3.84	2.42	3.33	2.51
22:6		1.02	1.40	1.07	1.54
24:0		0.16	0.37	0.18	0.13
24:1		0.08	–	0.06	0.04
U/S [c]		1.58	1.51	1.90 [a]	1.54 [b]
P/S		0.72	0.62	1.03 [a]	0.66 [b]

[ab] Values for a fatty acid or ratio with different superscripts for a P/S group are significantly different, thus indicating a change in the mole percent of that fatty acid or the mole percent ratio when fat intake is lowered from 40 to 20% of energy. Superscripts are omitted when there are no significant effects.

[c] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated fatty acids.

lipids that tend to reduce fluidity. Monolayers consisting of saturated phospholipids such as distearoylphosphatidyl choline or monolayers consisting of egg lecithin with added cholesterol bound less apoprotein. Analysis of their adsorption data suggested that lateral compressibility of a lipid monolayer is a major determinant of the extent of apolipoprotein adsorption. In their model the protein penetrates into the interface to occupy space made available by concomitant compression of phospholipid molecules. Hence the differences in surface lipid composition and packing that exist among various types of lipoprotein particles could be crucial in determining the conformation and reactivity of apoproteins and in controlling the distribution and transfer of apolipoproteins among various lipoprotein classes in vivo.

Paul et al. [48] discussed mechanisms of PUFA induction of hypocholesterolemia and postulated that a more fluid core in HDL may facilitate cholesteryl ester internalization into HDL. There have been several studies showing some changes in the fine structure of lipoprotein particles in coronary heart disease (CHD) patients. Ruuge et al. [53] reported a disturbance in cholesterol uptake function in hypoalphalipoproteinemia. When HDL cholesterol content changed, there were specific structural modifications in HDL_2 and HDL_3 which affect the ability of these molecules to incorporate spin-labeled fatty acids and steroids. HDL_2 and HDL_3 from patients with coronary atherosclerosis were reported [54] as having higher microviscosities associated with lower phosphatidylcholine/sphingomyelin ratios. Spin label data [55] demonstrated alterations in the lipid environment of HDL_2 and HDL_3 from CHD patients. It is to be expected that modifications of lipid structures and lipid-protein interactions as evidenced with these HDL fractions would impinge upon their interactions with vessel walls and thus contribute to atherogenesis.

The establishment of a direct clinical relation-

TABLE 12

EFFECT OF DIETARY FAT REDUCTION ON HDL CHOLESTERYL ESTER FATTY ACYL COMPOSITION, FOLLICULAR-PHASE DATA (FATTY ACID MOL PERCENTAGES AND U/S AND P/S RATIOS ARE MEAN VALUES)

Acid	P/S:	0.3		1.0	
	% Energy:	40.0	20.0	40.0	20.0
12:0		0.11	0.02	0.02	0.01
14:0		1.65	1.65	1.10	1.35
15:0		0.75	0.71	0.57	0.64
16:0		31.60 [b]	36.75 [a]	31.84 [b]	36.92 [a]
16:1		5.21 [b]	6.69 [a]	4.48 [b]	5.48 [a]
18:0		4.50	4.89	5.02	5.83
18:1		27.22	30.24	24.74	26.99
18:2		23.05 [a]	13.80 [b]	24.76 [a]	17.51 [b]
18:3		0.79	0.83	0.99	0.94
20:0		0.37	0.53	0.23	0.39
20:3		0.18	0.13	0.19	0.15
20:4		0.56 [a]	0.35 [b]	0.75	0.54
20:5		0.32	0.31	0.39	0.26
22:5		2.18	1.67	2.99	1.68
22:6		1.32	1.19	1.70	1.07
24:0		0.11 [b]	0.20 [a]	0.15	0.22
24:1		0.09	0.05	0.06	0.04
U/S [c]		1.59 [a]	1.29 [b]	1.62 [a]	1.31 [b]
P/S		0.75 [a]	0.43 [b]	0.85 [a]	0.54 [b]

[ab] Values for a fatty acid or ratio with different superscripts for a P/S group are significantly different, thus indicating a change in the mole percent of that fatty acid or the mole percent ratio when fat intake is lowered from 40 to 20% of energy. Superscripts are omitted when there are no significant effects.

[b] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated fatty acids.

TABLE 13

EFFECT OF DIETARY FAT REDUCTION ON HDL CHOLESTERYL ESTER FATTY ACYL COMPOSITION, LUTEAL-PHASE DATA (FATTY ACID MOL PERCENTAGES AND U/S AND P/S RATIOS ARE MEAN VALUES)

Acid	P/S:	0.3		1.0	
	% Energy:	40.0	20.0	40.0	20.0
12:0		0.01	0.01	0.01	–
14:0		1.78	1.74	1.23	1.18
15:0		0.82	0.70	0.61	0.45
16:0		35.81	35.51	33.58	33.89
16:1		4.91 [b]	6.31 [a]	4.25 [b]	5.52 [a]
18:0		7.09	5.06	5.28	4.27
18:1		25.05 [b]	29.98 [a]	24.05 [b]	28.58 [a]
18:2		15.67	16.70	22.89	21.73
18:3		0.84	0.81	0.95	0.80
20:0		0.34	0.33	0.30	0.30
20:3		0.19	0.15	0.16	0.14
20:4		0.98	0.36	0.70 [a]	0.36 [b]
20:5		0.16	0.30	0.29	0.17
22:5		3.55 [a]	0.88 [b]	3.74 [a]	1.34 [b]
22:6		2.28 [a]	0.91 [b]	1.71	1.06
24:0		0.27	0.18	0.17	0.17
24:1		0.26	0.08	0.09	0.06
U/S [c]		1.21	1.36	1.53	1.75
P/S		0.53	0.49	0.80	0.87

[ab] Values for a fatty acid or ratio with different superscripts for a P/S group are significantly different, thus indicating a change in the mole percent of that fatty acid or the mole percent ratio when fat intake is lowered from 40 to 20% of energy. Superscripts are omitted when there are no significant effects.

[c] U/S and P/S values were calculated by dividing the total moles unsaturated (U) or polyunsaturated (P) fatty acids by the total moles saturated fatty acids.

ship between decreases in lipoprotein fluidity and coronary heart disease is difficult, since lipoprotein fluidity is easily changed as diet is modified; however, the potential harmful cardiovascular effects for an individual would be the result of long-term dietary inadequacies. It is evident from this study that the reduction of dietary fat led to physical chemical changes in lipoprotein lipids which may affect lipid-protein interactions in a manner that ultimately protects against atherosclerosis.

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References

1 Adlersberg, D. and Schaffer, L., Age, sex, serum lipids and coronary atherosclerosis, J. Am. Med. Assn., 162 (1956) 619.

2 James, T., Post, H. and Smith, F., Myocardial infarction in women, Ann. Intern. Med., 43 (1955) 153.

3 Weinred, H., German, E. and Rosenberg, B., A study of myocardial infarction in women, Ann. Intern. Med., 46 (1957) 285.

4 Kannel, W., Hyortland, M., McNamara, P. and Gordon, T., Menopause and risks of cardiovascular disease – the Framingham study, Ann. Intern. Med., 85 (1976) 447.

5 Brook, J.G., Aviram, M., Oettinger, M. and Sharf, M., The effect of oestrogen implants on high density lipoprotein and its subfractions in women in their premature menopause, Maturitas, 4 (1982) 257.

6 Miller, G.J. and Miller, N.E., Plasma high density lipoprotein concentration and development of ischaemic heart disease, Lancet, 1 (1975) 16.

7 Lewis, B., Chait, A., Wootton, I.D.P., Oakley, C.M., Krikler, D.M., Sigurdsson, G., February, A., Maurer, B. and Birk-

head, J., Frequency of risk factors for ischaemic heart disease in a healthy British population, Lancet, 1 (1974) 141.
8 Rifkind, B., Tamir, I. and Heiss, G., Preliminary high density lipoprotein findings. The lipid research clinics program, In: High Density Lipoprotein and Atherosclerosis, Gotto, A.M. Jr., Miller, N.E. and Oliver, M.F. (Eds.), Elsevier/North-Holland Biomedical Press, Amsterdam, 1979, p. 109.
9 Lewis, B., The Hyperlipidaemias, Blackwell Scientific Publications, Oxford, 1976, p. 162.
10 Oliver, M. and Boyd, G., The influence of the sex hormone on the circulating lipids and lipoproteins in coronary sclerosis, Circulation, 13 (1973) 82.
11 Tikkanen, M., Nikkila, E.A. and Varlainen, E., Natural oestrogen as an effective treatment for type II hyperlipoproteinemia in postmenopausal women, Lancet, 2 (1978) 490.
12 Semmens, J., Rouse, I., Beilin, L.J. and Masarei, J.R.L., Relationship of plasma HDL-cholesterol to testosterone, estradiol and sex-hormone-binding globulin levels in men and women, Metabolism, 32 (1983) 428.
13 Berlin, E., Khan, M.A., Henderson, G.R. and Kliman, P.G. Influence of age and sex upon composition and lipid fluidity in miniature swine plasma lipoproteins, Atherosclerosis, 54 (1985) 187.
14 Berlin, E., Judd, J.T., Marshall, M.W. and Kliman, P.G., Dietary linoleate increases fluidity and influences chemical composition of plasma low density lipoprotein in adult men, Atherosclerosis, 66 (1987) 215.
15 Kohlmeier, M., Stricker, G. and Schief, G., Influences of "normal" and "prudent" diets on biliary and serum lipids in healthy women, Am. J. Clin. Nutr., 42 (1985) 1201.
16 Weisweiler, P., Janetschek, P., Schwandt, P., Influence of polyunsaturated fats and fat restriction on serum lipoproteins in humans, Metabolism, 34 (1985) 83.
17 Brussard, J.H., Katan, M.B., Groot, P.H.E., Havekes, L.M. and Hautvast, J.G.A.J., Serum lipoproteins of healthy persons fed a low-fat diet or a polyunsaturated fat diet for three months, Atherosclerosis, 42 (1982) 205.
18 Brussard, J.H., Dallinga-Thie, G., Groot, P.H.E. and Katan, M.B., Effects of amount and type of dietary fat on serum lipids, lipoproteins and apolipoproteins in man, Atherosclerosis, 36 (1980) 515.
19 Shepherd, J., Stewart, J.M., Clark, J.G. and Carr, K., Sequential changes in plasma lipoproteins and body fat composition during polyunsaturated fat feeding in man, Br. J. Nutr., 44 (1980) 155.
20 Jones, D.Y., Judd, J.T., Taylor, P.R., Campbell, W.S. and Nair, P.P., Menstrual cycle effect on plasma lipids, Metabolism, 37 (1988) 1.
21 Jones, D.Y., Judd, J.T., Taylor, P.R., Campbell, W.S., and Nair, P.P., Influence of dietary fat on menstrual cycle and menses length, Human Nutr: Clin. Nutr., 41C (1987) 341.
22 Bhathena, S.J., Berlin, E., Judd, J.T., Nair, P.P., Kennedy, B.W., Jones, J., Smith, P.A., Jones, Y., Taylor, P.R., Campbell, W. and Harris, T.C., Hormones regulating lipid and carbohydrate metabolism in premenopausal women: modulation by dietary lipids, Am. J. Clin. Nutr., in press.
23 Bhathena, S.J., Berlin, E., Judd, J.T., Jones, J., Kennedy, B.W., Smith, P.M., Jones, D.Y., Taylor, P.R., Campbell, W.S., Blanchard, S., and Nair, P.P., Dietary fat and menstrual cycle effects on the erythrocyte ghost insulin receptor in premenopausal women, Am. J. Clin. Nutr., in press.
24 Taylor, P.R., Schiffman, M.H., Jones, D.Y., Judd, J., Schatzkin, A., Nair, P.P., Van Tassell, R., and Block, G., Relation of changes in amount and type of dietary fat to fecapentaenes in premenopausal women, Mutation Res. 206 (1988) 3.
25 Metropolitan Life Insurance Company: 1983 Metropolitan height and weight tables, Stat. Bull., 64 (1983) 1.
26 Recommended Dietary Allowances, Committee on Dietary Allowances, Food and Nutrition Board, Commission on Life Sciences, National Research Council. 9th edition, Washington, DC, National Academy Press, 1980.
27 Haning, R., Orczyk, G.P., Caldwell, B.V., and Behrman, H.R., Plasma estradiol, estrone, estriol, and urinary estriol glucuronide. In: Methods of Hormone Radioimmunoassay, 2nd edition, Jaffe, B.M. and Behrman, H.R. (Eds.), Academic Press, New York, 1979, pp. 675–700.
28 Orczyk, G.P., Hichens, M., Arth, G., and Behrman, H.R., Progesterone and 20α-Dihydroprogesterone. In: Methods of hormone Radioimmunoassay, 2nd edition, Jaffe, B.M. and Behrman, H.R. (Eds.), Academic Press, New York, 1979, pp. 701–713.
29 Hatch, F.T. and Lees, R.S., Practical methods for plasma lipoprotein analysis, Adv. Lipid Res., 6 (1968) 1.
30 Lindgren, F.T., Serum lipoproteins – Isolation and analysis with the preparative and analytical ultracentrifuge. In: Fundamentals of Lipid Chemistry, Burton, R.M. and Guerra, F.C. (Eds.), BI-Science Publications, Webster Groves, MO, 1974, p. 475.
31 Bronzert, T.J. and Brewer, H.B., New micromethod for measuring cholesterol in plasma lipoprotein fractions, Clin. Chem., 23 (1977) 2089.
32 Shinitzky, M. and Barenholz, Y., Fluidity parameters of lipid regions determined by fluorescence, Biochim. Biophys. Acta, 515 (1978) 367.
33 Azumi, T. and McGlynn, S.P., Polarization of the luminescence of phenanthrene, J. Chem. Phys., 37 (1962) 2413.
34 Chen, R.F. and Bowman, R.L., Fluorescence polarization measurement with ultraviolet-polarizing filters in a spectrofluorometer, Science, 147 (1965) 729.
35 Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Fu, P.C., Enzymatic determination of total serum cholesterol, Clin. Chem., 20 (1974) 470.
36 Bucolo, G. and David, H., Quantitative determination of serum triglycerides by the use of enzymes, Clin. Chem., 19 (1973) 476.
37 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the Folin phenol reagent, J. Biol. Chem., 193 (1951) 265.
38 Bartlett, G.R., Phosphorus assay in column chromatography, J. Biol. Chem., 234 (1959) 466.

39 Matusik, E.J., Reeves, V.B. and Flanagan, V.P., Determination of fatty acid methyl esters. Elimination of tissue-derived contamination and artefacts and preparation of a support-coated acid modified polyester liquid phase, Anal. Chim. Acta, 166 (1984) 179.
40 Sperry, W.M. and Brand, F.C., The determination of total lipids in blood serum, J. Biol. Chem., 213 (1955) 69.
41 Rouser, G., Kritshevsky, G., and Yamamoto, A., Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids. In: Lipid Chromatographic Analysis, Vol. 1, Marinetti, G.V., (ed.), Marcel Dekker, Inc., New York, 1967, pp. 99–162.
42 Helwig, J.T. and Council, K.A. (Eds.), SAS User's Guide, SAS (Statistical Analysis System) Institute, Cary, NC, 1979.
43 Hodges, R.E. and Krehl, W.A., The role of carbohydrates in lipid metabolism, Am. J. Clin. Nutr., 17 (1965) 334.
44 Dumaswala, U.J., Dumaswala, R.U. and Venkataraman, A., The relative effect of dietary fats and carbohydrates on lipid metabolism in the albino rat, Ital. J. Biochem., 25 (1976) 289.
45 Deshaies, J., Plasma lipoprotein cholesterol and triglycerides and lipoprotein lipase activity in epididymal white adipose tissue of rats fed high sucrose or high corn oil diets, Can. J. Physiol. Pharmacol., 64 (1986) 885.
46 Hay, R.V., Pottenger, L.A., Getz, G.S. and Wissler, R.W., Degradation of ^{125}I-labelled serum low density lipoprotein in normal and estrogen-treated male rats, Biochem. Biophys. Res. Commun., 44 (1971) 1471.
47 Kovanen, P.T., Brown, M.S. and Goldstein, J.L., Increased binding of low density lipoprotein to liver membrane from rats treated with 17α-ethinyl estradiol, J. Biol. Chem. 254 (1979) 11367.
48 Paul, R., Ramesha, C.S. and Ganguly, J., On the mechanisms of the hypocholesterolemic effects of polyunsaturated lipids, Adv. Lipid Res., 17 (1980) 155.
49 Berlin, E and Young, C., Influence of dietary fats on the fluidity of the lipid domains of rabbit plasma lipoproteins, Atherosclerosis, 35 (1980) 229.
50 Berlin, E. and Young, C., Effects of fat level, feeding period, and source of fat on lipid fluidity and physical state of rabbit plasma lipoproteins, Atherosclerosis, 48 (1983) 15.
51 Soutar, A., Does dietary fat influence plasma lipoprotein structure? Nature, 273 (1978) 11.
52 Ibdah, J.A., and Phillips, M.C., Effects of lipid composition and packing on the adsorption of apolipoprotein A-I to lipid monolayers, Biochemistry, 27 (1988) 7155.
53 Ruuge, E.K., Gerasimova, E.N., Gorshkova, I.N., and Perova, N.V., A study of high density lipoprotein subclasses by spin labeling, In: High Density Lipoproteins and Atherosclerosis, Medicina, Moscow, 1983, p. 103.
54 Fam-Thi-Mai, Torkhovskaya, T.I., Oserova, I.N., Polesski, V.A., Kurdanov, Kh. A., and Gerasimova, E.N., Alteration in the structure of high density lipoprotein subfractions from patients with ischemic heart disease, Vopr. Med. Chim., 5 (1981) 701.
55 Panasenko, O.M., Asisova, O.A., Torkhovskaya, T.I., and Dudaev, V., A study of blood plasma lipoprotein structural organization in patients with coronary heart disease by spin labelling, Vopr. Med. Chim., 30 (1984) 40.